

# Effect of Wintering Duration and Temperature on Survival and Emergence Time in Males of the Orchard Pollinator *Osmia lignaria* (Hymenoptera: Megachilidae)

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**ABSTRACT** We measured winter survival, emergence time following incubation at 20°C, and postemergence longevity in males of the orchard pollinator *Osmia lignaria* Say exposed to 25 artificial wintering treatments differing in duration (30, 90, 150, 210, and 270 d) and temperature (0, 4, 7, 10, and 13°C). For all temperatures, survival was highest at 90 d of wintering. Temperatures  $\geq 10^\circ\text{C}$  were unsuitable for wintering *O. lignaria* populations even for short periods. At 7°C, bees showed increased mortality, signs of excessive fat body depletion, and decreased longevity when wintered for  $>150$  d. Wintering durations  $\geq 210$  d required temperatures  $\leq 4^\circ\text{C}$  for acceptable survival. Time to emerge following incubation at 20°C decreased with increasing wintering duration and wintering temperature. Many bees emerged before incubation when wintered for long periods at the warmest temperatures. Postemergence longevity was highest when bees were wintered at 0°C for 210 d. Managing the activity of *O. lignaria* to coincide with fruit tree bloom initiation is much more easily accomplished with the use of populations exhibiting short emergence times. Several wintering treatments that resulted in acceptable survival and longevity (150 d at 0, 4 and 7°C, and 210–270 d at 0 and 4°C), produced mean emergence times  $\leq 5$  d. Our results can be used to establish appropriate wintering regimes for *O. lignaria* populations managed to pollinate orchard crops differing in bloom time (from almonds in February to apples in May), and therefore, allowing for shorter or longer wintering durations.

**KEY WORDS** *Osmia lignaria*, wintering, temperature, mortality, emergence, orchard pollination

THE SOLITARY BEE *Osmia lignaria* Say is a North American species that flies in spring, nests gregariously in preestablished cavities, and shows a strong preference to collect pollen from Rosaceae (Torchio 1976, 1981; Bosch and Kemp 1999; Bosch et al. 2000). Its pollinating efficacy on fruit tree flowers is very high, contacting the anthers and stigmas on virtually every visit. For these reasons, methods to manage *O. lignaria* as an orchard pollinator have been developed in the USA (Torchio 1976, 1982, 1985; Bosch and Kemp 1999, 2001; Bosch et al. 2000). As with any other insects managed to assist human agriculture, the development of appropriate rearing methods for this valuable pollinator depends on sufficient knowledge of its life cycle and basic developmental biology (Bosch and Kemp 2002). *Osmia lignaria* is protandrous, obligatorily univoltine, and winters as a cocooned dormant adult (Rust 1974, Torchio 1989). Current studies investigating respiration rates and intermediary metabolism of wintering *O. lignaria* adults (W.P.K., J.B., and B. Dennis, unpublished; W.P.K., J.B., unpublished) indicate that this

dormancy is diapause-mediated (*sensu* Tauber et al. 1986).

Development from egg to adult takes place in darkness within the nest. Eggs hatch a few days after oviposition in spring, and immature development proceeds through five instars (Torchio 1989). The last instar spins a cocoon and undergoes a summer dormant period (also probably diapause-mediated), followed by pupation. In nature, pupae turn into adults in late summer (Torchio 1989, Bosch and Kemp 2000). Newly formed adults shed the pupal cuticle and remain in the cocoon until emergence in the following spring (Torchio 1989). These cocooned adults require cold exposure followed by increasing warm temperatures to emerge in the following spring, and individuals wintered as prepupae or pupae do not survive (Bosch and Kemp 2000). Therefore, we use the term “wintering” to define the fall-winter period during which cocooned adults are exposed to low temperatures, and “incubation” to define the spring period during which wintered cocooned adults are exposed to warm temperatures that elicit emergence. *Osmia lignaria* populations reared artificially (at controlled temperatures) are routinely exposed to temperatures  $\approx 22^\circ\text{C}$  during development (spring-summer), followed by cooling at 3–5°C during wintering (Torchio 1985, Bosch and Kemp 2001). The effect of different

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temperature treatments on *O. lignaria* spring-summer development rates and survival has been reported in some detail (Bosch and Kemp 2000, Bosch et al. 2000), but wintering treatments have received less attention.

Studies on natural populations indicate that wintering duration and temperature affect not only winter survival, but also emergence timing of *O. lignaria* (Rust 1995, Dogterom 1999). Populations exposed to short wintering (e.g., <100 d) exhibit protracted emergence when compared with bees wintered for >180 d (Bosch et al. 2000). Additionally, bees wintered at 7°C, exhibited shorter emergence times than bees wintered at 4°C for the same duration (Bosch et al. 2000). Because fruit trees are normally in bloom for only 2–3 wk, *O. lignaria* populations must be managed for rapid and synchronized emergence to be effectively used for orchard pollination (Bosch and Kemp 2001). In this study, we measured winter survival, emergence times, and longevity (as a measure of vigor) in *O. lignaria* populations exposed to a variety of artificial wintering treatments that differed in duration and temperature. We were interested in determining how these two variables interact to influence bee fitness and emergence timing, and we used this information to establish appropriate wintering regimes (those yielding low mortality and synchronized emergence times) for *O. lignaria* populations used in orchard pollination. We believe that this information will be useful in improving attempts to synchronize bee emergence with bloom initiation in various fruit trees, ranging from almonds in February to apples in May.

### Materials and Methods

**Experimental Population.** We used the progeny of an *O. lignaria* population released in a cherry orchard in North Ogden, UT, in 2000. Nests obtained in this orchard were brought to the laboratory on 18 May and held at a constant 22°C and ≈70% RH for the spring-summer development period. Every 2 wk, a sample of nests was examined via X-ray (Stephen and Undurraga 1976), and when all bees on the X-ray plates had reached adulthood (16 August), nests were dissected to select 750 cocooned males; cocoons containing males are smaller and closer to the nest entrance than cocoons containing females (Torchio 1989, Bosch and Kemp 2001). These cocoons were placed in clear gel capsules, labeled by nest number, and distributed among 25 wintering treatments, described below, so that no treatment received more than one cocoon from the same nest. There were 30 males per treatment. No female individuals were used, because previous studies showed that both sexes respond similarly to temperature regimes (Bosch and Kemp, 2000; J.B., W.P.K., unpublished).

**Wintering Treatments and Incubation.** Upon 100% adulthood (16 August), cocoons were held at 22°C for 15 d, and then cooled to 13°C for 5 d before transfer to their respective wintering treatments (5 September). Wintering treatments combined five durations (30, 90, 150, 210, and 270 d) and five constant tem-

**Table 1.** Frequency and percentage of male *O. lignaria* emerging before completion of their assigned wintering treatment. Sample sizes are ≈30 individuals per treatment

Wintering duration	Wintering temperature				
	0°C	4°C	7°C	10°C	13°C
30 d	0	0	0	0	0
90 d	0	0	0	0	0
150 d	0	0	1 (3.3%)	15 (50.0%)	17 (63.0%)
210 d	0	0	22 (73.3%)	30 (100%)	28 (93.3%)
270 d	0	0	22 (73.3%)	30 (100%)	28 (93.3%)

peratures (0, 4, 7, 10, and 13°C). Upon completion of each wintering treatment, cocoons were placed individually in glass vials and incubated at a constant 20°C. Cocoons were monitored daily for emergence and death after emergence. Throughout the study, relative humidity in the temperature cabinets used was ≈70%. Cocoons were kept in darkness at all times because, in natural conditions, cocooned adults within their nests are not exposed to light.

**Survival, Emergence Time, and Longevity.** We defined survival as the percentage of individuals emerging completely from the cocoon following incubation at 20°C, emergence time as the interval between incubation date and emergence date, and longevity without feeding (a measure of vigor, Bosch and Kemp 2000) as the interval between emergence date and death date. Some bees assigned to longer wintering periods and higher temperatures emerged before the completion of their assigned wintering treatment (Table 1), before the scheduled incubation at 20°C. These winter emerging bees were considered not viable, and thus, were not included in subsequent emergence time and longevity analyses.

The frequency of extreme values (0 and 100%) observed in the survival data prevented logistic or categorical data modeling approaches from converging satisfactorily. As an alternative approach, we constructed two sets of tables: (1) wintering duration (30, 90, 150, 210, 270 d) by outcome (alive or dead), while controlling for wintering temperature (0, 4, 7, 10, 13°C); and (2) wintering temperature by outcome, while controlling for wintering duration, and tested whether outcome was independent of duration and temperature, respectively, using a  $\chi^2$  likelihood ratio test statistic (PROC FREQ; SAS Institute Inc. 1999).

As noted above, in some of the warmer and longer wintering treatments a significant number of bees emerged before completion of their assigned treatment (Table 1). As a result, we could not conduct a  $5 \times 5$  analysis of variance (ANOVA) with emergence time and longevity data as originally planned. Instead, using only those treatments where overwhelming winter emergence ( $\geq 50\%$ ) was not encountered, we conducted two mixed-model ANOVAs (PROC MIXED; SAS Institute Inc. 1999): (1) durations 30, 90, and 150 d  $\times$  temperatures 0, 4, 7, 10, and 13°C; and (2) temperatures 0 and 4°C  $\times$  durations 30, 90, 150, 210, 270 d. The mixed model specified heterogeneous variances for duration levels, which addressed the observed positive relationship between means and variances.

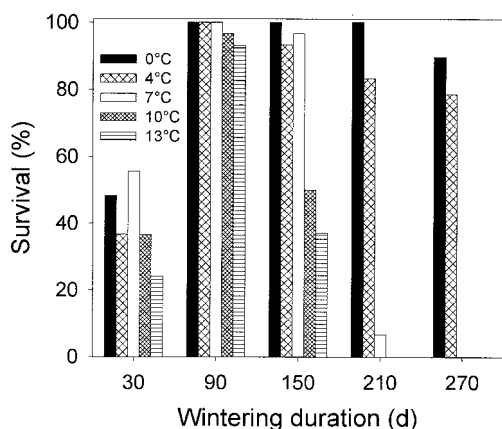


Fig. 1. Percent survival of adult male *O. lignaria* wintered under various treatments (durations: 30, 90, 150, 210, and 270 d; temperatures: 0, 4, 7, 10, and 13°C), and incubated at 20°C to emergence. Sample sizes are  $\approx 30$  individuals per treatment.

As the assumption of equal variances was not violated for longevity, we used PROC GLM (SAS Institute Inc. 1999) to conduct two ANOVAs: (1) durations 30, 90, and 150 d  $\times$  temperatures 0, 4, 7, 10, and 13°C; and (2) temperatures 0 and 4°C  $\times$  durations 30, 90, 150, 210, 270 d.

**Fat Body Depletion.** Previous studies showed that apparent fat body condition in wintered adult *O. lignaria* could be assessed through X-ray, and that bees showing signs of fat body depletion were less vigorous than bees with well-developed fat bodies (Bosch et al. 2000, Bosch and Kemp 2001). X-ray photographs of bees with fat body depletion show dark (empty) areas in the abdomen, in contrast to bees with abundant fat body, whose abdomens appear totally white (full) (see Bosch et al. 2000, Bosch and Kemp 2001). We used this method to assess the apparent physiological condition at the completion of wintering for bees assigned to all 150 and 210 d treatments. We used a likelihood ratio  $\chi^2$  test statistic (PROC FREQ; SAS Institute Inc. 1999) to compare differences in fat body condition upon completion of wintering across wintering temperatures for wintering durations of 150 and 210 d separately.

## Results

**Survival.** Percent survival of *O. lignaria* was low when bees were wintered for only 30 d, but increased to levels between 90–100% when wintering was extended to 90 d (Fig. 1). Likelihood ratio  $\chi^2$  results of comparisons across wintering temperatures while controlling for wintering duration were significant for wintering durations  $\geq 150$  d (150 d  $\chi^2 = 66.55$ , df = 4,  $P < 0.0001$ ; 210 d  $\chi^2 = 157.49$ , df = 4,  $P < 0.0001$ ; 270 d  $\chi^2 = 137.33$ , df = 4,  $P < 0.0001$ ). Survival declined as wintering duration was extended beyond 90 d, rapidly at the warmer temperatures and more slowly at the colder temperatures (Fig. 1). Thus, bees could be

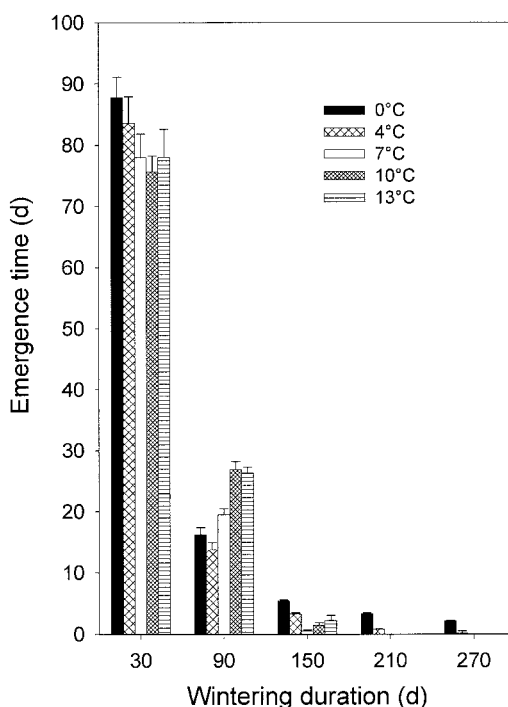


Fig. 2. Mean emergence time  $\pm$  SE (days from incubation at 20°C to emergence) of adult male *O. lignaria* wintered under various treatments. Sample sizes are 8–30 individuals per treatment.

wintered without significant reductions in survival for up to 210 d at 0°C, but only for 150 d at 7°C, and for 90 d at 10–13°C (Fig. 1). Likelihood ratio  $\chi^2$  results for comparisons across wintering durations, while controlling for wintering temperature, were all significant (0°C  $\chi^2 = 49.58$ , df = 4,  $P < 0.0001$ ; 4°C  $\chi^2 = 42.80$ , df = 4,  $P < 0.0001$ ; 7°C  $\chi^2 = 141.73$ , df = 4,  $P < 0.0001$ ; 10°C  $\chi^2 = 107.36$ , df = 4,  $P < 0.0001$ ; 13°C  $\chi^2 = 95.33$ , df = 4,  $P < 0.0001$ ).

**Emergence.** Emergence time declined with wintering duration, with the greatest improvements (reductions in mean and variance) occurring when wintering duration increased from 30 to 90 d (Fig. 2). ANOVA results for the mixed-model computed for all five wintering temperatures (0, 4, 7, 10, and 13°C) and the three wintering durations (30, 90, and 150 d) yielding no or little winter emergence (see Table 1) revealed no significant differences in emergence times across wintering temperature ( $F = 1.82$ ; df = 4, 295;  $P = 0.125$ ). However, there were significant differences across wintering durations ( $F = 1447.38$ ; df = 2, 295;  $P < 0.0001$ ), as well as a wintering temperature  $\times$  duration interaction ( $F = 17.20$ ; df = 8, 295;  $P < 0.0001$ ). Mixed-model results for a second ANOVA computed for all five wintering durations and the two wintering temperatures (0 and 4°C) yielding no winter emergence (see Table 1) revealed significant differences in emergence times between temperatures ( $F = 5.29$ ; df = 1, 230;  $P = 0.022$ ), and across durations ( $F = 368.40$ ; df = 4, 230;  $P < 0.0001$ ), with no signif-

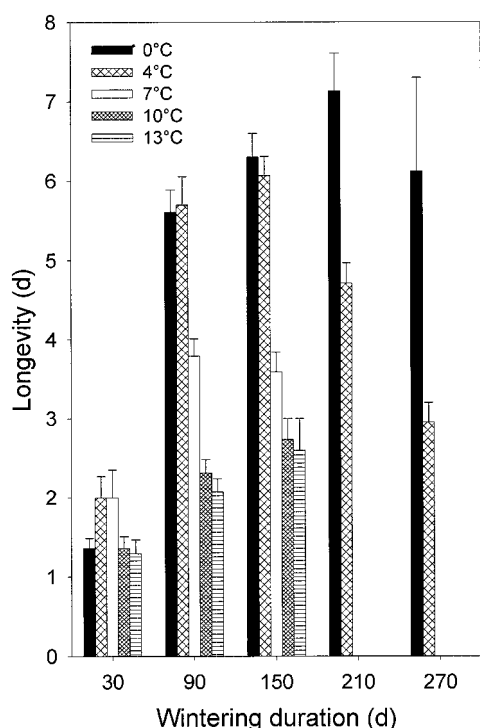


Fig. 3. Mean longevity  $\pm$  SE (days from emergence to death at 20°C) of male *O. lignaria* wintered under various treatments. Sample sizes are 8–30 individuals per treatment.

icant temperature  $\times$  duration interaction ( $F = 1.41$ ;  $df = 4, 230$ ;  $P = 0.231$ ).

**Longevity.** The results for *O. lignaria* longevity were similar to those observed for survival, but with maximum values shifted to longer ( $\approx 150$  d) wintering durations (Fig. 3). Longevity clearly declined as wintering temperatures increased (Fig. 3). ANOVA results for the mixed-model computed for all five wintering temperatures and three wintering durations (30, 90, and 150) revealed significant differences in longevity across wintering temperature ( $F = 44.11$ ;  $df = 4, 295$ ;  $P < 0.0001$ ), and wintering duration ( $F = 81.93$ ;  $df = 2, 295$ ;  $P < 0.0001$ ), as well as a wintering temperature  $\times$  duration interaction ( $F = 8.40$ ;  $df = 8, 295$ ;  $P < 0.0001$ ). Results for a second ANOVA including all five wintering durations and temperatures 0 and 4°C also revealed significant differences in longevity across wintering temperatures ( $F = 21.55$ ;  $df = 1, 230$ ;  $P < 0.0001$ ), and wintering durations ( $F = 40.87$ ;  $df = 4, 230$ ;  $P < 0.0001$ ), with a wintering temperature  $\times$  duration interaction ( $F = 11.94$ ;  $df = 4, 230$ ;  $P < 0.0001$ ).

**Fat Body Depletion.** The percentages of *O. lignaria* from the 150- and 210-d treatments with signs of fat body depletion are shown in Table 2. Apparent fat body depletion was obviously greater among bees wintered for 210 d, and, in general, increased with rising wintering temperature (likelihood ratio  $\chi^2$  for 150 d = 51.13,  $df = 4$ ,  $P < 0.0001$ ; likelihood ratio  $\chi^2$  for 210 d = 16.56,  $df = 2$ ,  $P < 0.0003$ ).

Table 2. Frequency and percentage of emerged male *O. lignaria* showing signs of fat body depletion after wintering. Sample sizes are 8–30 individuals per treatment

Wintering duration	Wintering temperature				
	0°C	4°C	7°C	10°C	13°C
150 d	2 (6.7%)	1 (3.3%)	3 (10.0%)	7 (46.7%)	12 (92.3%)
210 d	17 (56.7%)	9 (30.0%)	8 (100%)	—	—

## Discussion

Cocooned *O. lignaria* adults require exposure to cold temperatures (wintering) followed by warm temperatures (spring incubation) for successful emergence in the spring. Populations exposed to longer wintering periods require less incubation to emerge than populations exposed to shorter wintering periods. However, populations wintered at warmer temperatures require less incubation to emerge. These results are consistent with findings in various winter-diapausing insects that require a period of low-temperature exposure, but whose diapause development proceeds faster at warmer than cooler temperatures (at least within certain temperature ranges; Danks 1987). Emergence of nonwintered *O. lignaria* adults (cocooned adults not exposed to cold temperatures during the winter) is extremely rare. In the closely-related species *O. cornuta* (Latreille), which also winters in the adult stage and flies in spring, only 2.7% individuals emerged after 1-d-wintering, and 9.2% after 2-wk-wintering (J.B. and W.P.K., unpublished). *Osmia lignaria* populations from northern Utah require a wintering period of  $\approx 90$  d for maximum survival at the temperatures tested. For longer wintering durations, survival decreases at all temperatures tested except at 0°C.

In nature, *O. lignaria* populations from northern Utah are exposed to winters much longer than 90 d. In a previous study, in which an *O. lignaria* population from northern Utah was wintered under natural conditions, hourly temperatures from 5 September to 2 April (210 d) averaged 2.44°C (SE = 0.11°C; range =  $-17.11$ – $29.08$ °C) (Bosch and Kemp 2000). Winter mortality of that population was 6.4%, with emergence peaking in mid April (Bosch and Kemp 2000). Bees wintered for 210 d in this study had 0% mortality when wintered at 0°C and 16.7% mortality when wintered at 4°C. The combination of natural- and artificial-wintering results suggests that optimal wintering temperature for *O. lignaria* populations from northern Utah is  $< 4$ °C. This is further supported by our longevity results, which, for 210-d-wintering-periods, show a sharp decline in vigor when wintering temperatures are increased from 0 to 4°C. Our results are similar to those of Irwin and Lee (2000), who found that in the goldenrod gall fly *Eurosta solidaginis* (Fitch), individuals wintered at mild temperatures (12°C) had increased body weight loss, reduced survival, and laid fewer eggs than individuals wintered at 0°C. Similarly, in the Coccinellid *Coleomegilla maculata lengi* Timberlake, lipid consumption throughout the winter increased with increasing wintering temperatures, and



survival was higher at intermediate ( $-0.5$ – $4^{\circ}\text{C}$ ) than at high ( $10$ – $20^{\circ}\text{C}$ ) or low ( $-10$  to  $-4^{\circ}\text{C}$ ) wintering temperatures (Jean et al. 1990).

Despite the high survival obtained in all 90-d-treatments, *O. lignaria* requires longer wintering periods for prompt emergence. Bees wintered for 90 d only emerged after inordinately long (14–27 d) periods of incubation at  $20^{\circ}\text{C}$ . During the first three months of wintering, respiration rates of *O. lignaria* adults in their cocoons are approximately half that observed at 215 wintering d (W.P.K., J.B., and B. Dennis, unpublished). Adults removed from their cocoons in the middle of the winter walk actively, but do not seem to be able to fly, and, unlike fully wintered adults, do not attempt to sip nectar from flowers (unpublished data). Thus, at 90-d-wintering, adult *O. lignaria* from northern Utah have fulfilled their physiological requirements for survival, but not for prompt emergence and activity. This pattern is consistent with prevalent models in the insect diapause literature that indicate different temperature requirements for diapause and postdiapause completion, respectively (Tauber et al. 1986, Danks 1987, Hodek and Hodková 1988). Respiration rates in wintering *O. lignaria* increase after  $\approx 90$  d of wintering, suggesting a possible transition from diapause to postdiapause at this time (W.P.K., J.B., and B. D., unpublished data).

Adequate winter management of *O. lignaria* populations used for orchard pollination should address not only survival and vigor, but also emergence timing. Because of the short blooming period (2–3 wk) of most orchard crops, only those winter treatments yielding the least variable and most reduced emergence times are applicable to managed populations. From this perspective, wintering at  $4^{\circ}\text{C}$  would provide better timing with bloom initiation than wintering at  $0^{\circ}\text{C}$ . Even differences in mean emergence time of 2–3 d should be considered relevant for several reasons. First, releasing *O. lignaria* populations too early with respect to orchard bloom results in lower female establishment (unless alternative floral resources are present near or within the orchard; Bosch and Kemp 2001). Second, in this protandrous species, mean female emergence follows male emergence by 2–3 d (Bosch and Kemp 2000). Lastly, *O. lignaria* females require, upon emergence, 2–3 d to mate and presumably complete ovary maturation, select a nesting cavity, and establish (Bosch and Kemp 2001).

The use of late-flying (April–May) *O. lignaria* populations for early blooming crops (e.g., February-blooming almonds) necessitates the reduction of wintering duration, and thus will result in protracted emergence periods. As shown in this study, populations can be wintered at warmer temperatures resulting in shortened emergence periods, but only for limited periods of time before bees start showing signs of fat body depletion, decreased vigor, and emergence of a significant proportion of the population during wintering. For example, *O. lignaria* populations from northern Utah cannot be wintered at  $7^{\circ}\text{C}$  for longer than 150 d, and temperatures of  $10$ – $13^{\circ}\text{C}$  are unsuitable even for 90 d. Preemergence periods of late-flying

populations forced to fly ahead of their natural seasonal cycle can also be shortened by shortening developmental periods, and therefore, increasing wintering duration (Bosch et al. 2000). In 2002, we used an approach that combined fast developmental rates and warm wintering temperatures. We reared a late-flying population (April–May) at  $14.27^{\circ}\text{C}$  (8:16 h thermoperiod), which allowed us to initiate wintering in early- instead of late-September. This population was wintered at  $4^{\circ}\text{C}$  until 7 January (approximately 1 mo before expected almond bloom), when it was transferred to  $7^{\circ}\text{C}$ . The first males started emerging in the  $7^{\circ}\text{C}$  cooler on 30 January, shortly before almond bloom (J.B. and W.P.K., unpublished data). Winter emergence (emergence within the wintering cooler) of a few males is a good indicator that a population is ready to emerge (will require little incubation) and is still vigorous. If bloom is delayed and winter emergence has started, wintering temperature can be lowered to delay further emergence for at least 1 mo without serious consequences to bee vigor (Bosch and Kemp 2001). If bloom is advanced, the population will require some additional incubation for complete emergence. A parallel study has addressed the effect of incubation treatments on *O. lignaria* emergence (J.B. and W.P.K., unpublished data), and practical methods to increase preemergence temperatures within the nesting shelter are currently under study (Bosch and Kemp 2001). Knowing wintering initiation dates and approximate blooming dates of the target crop and, therefore, approximate wintering duration available, our results herein will facilitate the selection of appropriate wintering temperatures that will result in high survival and vigor, as well as predictable emergence patterns.

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